

We claim:

~~1. A method for determining the concentration of a specific lipoprotein, an apolipoprotein, or lipid associated with a specific lipoprotein, in a biological sample comprising:~~

~~immersing into the sample a solid phase material having immobilized thereon antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein;~~

~~allowing the antibody molecules time to bind to the lipoprotein or apolipoprotein in the sample;~~

~~removing the solid phase material containing the immobilized antibody molecules; and~~

~~determining the amount of lipoprotein, apolipoprotein, or lipid associated with a lipoprotein bound by the immobilized antibody molecules.~~

~~2. The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with a lipoproteins selected from the group consisting of HDL, LDL, VLDL, and combinations thereof.~~

~~3. The method of claim 2 wherein the antibody is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and antibody fragments.~~

~~4. The method of claim 3, wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB<sub>3</sub>CB<sub>3</sub> ATCC designation number HB 11612.~~

~~5. The method of claim 3, wherein the antibody is a recombinant anti-LDL RCB<sub>3</sub>M<sub>1</sub>D<sub>4</sub> ATCC designation number 69602.~~

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~~6. The method of claim 1 wherein the amount of lipoprotein, apolipoprotein lipid is determined by staining of the material bound to the immobilized antibody using a lipid stain.~~

7. The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8. The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

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~~9. The method of claim 6, further comprising antibody immunoreactive with apolipoprotein which is coupled to a protein stain and used to stain lipoprotein in the sample, prior to immersing into the sample the immobilized antibodies which then bind to the stained antibody bound apolipoprotein.~~

10. The method of claim 1, wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

~~12. A method of determining the concentration of an apolipoprotein in a biological sample comprising:~~

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~~mixing an antibody immunoreactive with a specific apolipoprotein into the sample;~~

~~allowing the antibody to bind to the apolipoprotein in the sample,~~

~~immersing into the mixture a second immobilized antibody immunoreactive with a second, distinct epitope of the apolipoprotein,~~

~~allowing the second immobilized antibody to bind to the apolipoprotein,~~

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detecting the presence of the  
 apolipoprotein bound by both both antibodies, and  
 determining the amount of  
~~apolipoprotein bound by both antibodies.~~

13. The method of claim 12 wherein the  
~~apolipoprotein is apolipoprotein Apo B-100.~~

14. The method of claim 12 for determining  
 the relative ratio of VLDL to HDL comprising  
 determining the amount of VLDL in a sample  
 based on the amount of Apo C-III present in the VLDL  
 in the sample by

providing immobilized Pan B antibody  
 which is characterized by an equal binding  
 and high affinity for all Apo B-containing  
 lipoproteins in human plasma,

providing soluble antibody  
 immunoreactive with Apo C-III having binding  
 affinity and specificity similar to XbA<sub>3</sub>,

mixing the soluble antibody reactive  
 with Apo C-III with the biological sample to  
 form complexes between the soluble antibody  
 and the Apo C-III containing lipoprotein  
 particles,

immersing the immobilized Pan B  
 antibody into the biological sample, and

determining the amount of Apo C-III  
 associated with Apo B, which is the amount  
 of Apo C-III present in VLDL in the sample;  
 and

determining the amount of HDL in a sample  
 based on the amount of Apo C-III present in the HDL in  
 the sample by

providing immobilized Apo A-I antibody  
 immunoreactive specifically with Apo A-I

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having a binding affinity and specificity similar to A1bD<sub>3</sub> and A1bE<sub>2</sub>,

providing soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA<sub>3</sub>,

mixing the soluble antibody reactive with Apo C-III with the biological sample to form complexes between the soluble antibody and the Apo C-III containing lipoprotein particles,

immersing the immobilized anti-Apo A-I antibody into the biological sample, and

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample.

15. The method of claim 12 for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in a sample based on the amount of Apo E present in the VLDL in the sample by

providing immobilized Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB<sub>1</sub> which binds to Apo E associated predominantly with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD<sub>3</sub> which binds to Apo E associated predominantly with HDL,

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adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles,

immersing the immobilized Pan B antibody into the biological sample, and determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample; and

determining the amount of HDL in a sample based on the amount of Apo E present in the HDL in the sample by

providing immobilized Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to AIB<sub>D</sub>,

providing a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB<sub>1</sub>, which binds to Apo E predominantly associated with VLDL, and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD<sub>3</sub>, which binds to Apo E predominantly associated with HDL,

adding the mixture of soluble antibodies reactive with Apo E to the biological sample to form complexes between the soluble antibodies and Apo E containing particles, and

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determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample.

16. The method of claim 12 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

providing immobilized anti-Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to A1bD<sub>5</sub>,

providing immobilized anti-Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB<sub>5</sub>;

mixing the soluble anti-Apo A-I antibody having a binding affinity and specificity similar to A1bE<sub>2</sub> to form complexes with both LPA-I and LPA-I:AI1;

immersing the immobilized anti-Apo A-I antibody into the biological sample and determining the quantity of Apo A-I associated with both LPA-I and LPA-II lipoprotein particles;

immersing the immobilized anti-Apo A-II antibody into the biological sample and determining the quantity of Apo A-I associated with the LPA-I:AI1.

17. A composition for determining the concentration of a lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein in a biological sample comprising:

a solid phase material having immobilized thereon antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein.

18. The composition of claim 17 further comprising a solid support to which the solid phase material is attached to form a dipstick.

19. The composition of claim 17 wherein the antibody is selected from the group consisting of

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monoclonal antibodies, recombinant antibodies, and antibody fragments.

20. The composition of claim 17 wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB<sub>3</sub>CB<sub>3</sub> ATCC designation number HB 11612.

21. The composition of claim 17 wherein the antibody is a recombinant anti-LDL RCB<sub>3</sub>M<sub>1</sub>D<sub>4</sub> ATCC designation number 69602.

22. The composition of claim 18 further comprising a solution containing molecules of a second soluble antibody immunoreactive with a second distinct epitope of the lipoprotein or apolipoprotein which is immunoreactive with the antibody molecules immobilized on the solid phase material.

23. The composition of claim 17 wherein the antibody molecules are immobilized to the solid phase material using avidin-biotin complexes.

24. The composition of claim 18 further comprising at least one internal standard comprising a known amount of a particular lipoprotein, lipoprotein lipid, or apolipoprotein immobilized on the solid phase material.

25. The composition of claim 17 wherein the solid phase material is selected from the group consisting of nitrocellulose, polyvinylidene difluoride, partially acid-hydrolyzed nylon, polystyrene, polypropylene, and paper.

26. The composition of claim 17 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

27. The composition of claim 17 for determining the relative ratio of VLDL to HDL comprising

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immobilized Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma, soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA<sub>3</sub>,

immobilized Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to AId<sub>5</sub> and AId<sub>2</sub>, and

soluble antibody immunoreactive with Apo C-III having binding affinity and specificity similar to XbA<sub>3</sub>.

28. The composition of claim 17 for determining the relative ratio of VLDL to HDL comprising

immobilized Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma, a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB<sub>1</sub> which predominantly binds to Apo E associated with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfD<sub>3</sub> which predominantly binds to Apo E in HDL,

immobilized Apo A-I antibody immunoreactive specifically with Apo A-I having a binding affinity and specificity similar to AId<sub>5</sub>, and

a mixture of soluble antibody immunoreactive with Apo E having binding affinity and specificity similar to EfB<sub>1</sub> which binds to Apo E predominantly associated with VLDL and soluble antibody immunoreactive with Apo E having binding affinity and

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specificity similar to Efd<sub>3</sub>, which predominantly binds to Apo E in HDL.

29. The composition of claim 17 for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

immobilized Apo A-I antibody which binds Apo A-I lipoproteins in human plasma having a binding affinity and specificity similar to AId<sub>3</sub>; and

immobilized Apo A-II antibody immunoreactive specifically with Apo A-II having a binding affinity and specificity similar to CdB<sub>3</sub>.

30. A method for making a composition comprising

immobilizing on a solid phase material antibody molecules immunoreactive with a specific lipoprotein or apolipoprotein, wherein the antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof.

31. The method according to claim 30 wherein the antibody molecule is specifically immunoreactive with LDL.

32. The method of claim 30 wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

33. An antibody molecule specifically immunoreactive with LDL that does not significantly cross-react with other lipoproteins in whole blood, blood plasma or blood serum, wherein the molecule is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and fragments thereof.

34. The antibody molecule of claim 33 wherein the antibody is the anti-LDL monoclonal

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antibody produced by the hybridoma cell line HB<sub>3</sub>CB<sub>3</sub>,  
ATCC designation number HB 11612.

35. The antibody molecule of claim 33  
wherein the antibody is a recombinant anti-LDL RCB<sub>3</sub>M<sub>1</sub>D<sub>4</sub>  
ATCC designation number 69602.

36. The antibody molecule of claim 33  
immobilized to a solid support.

37. The antibody molecule of claim 36  
wherein the support is a resin for purification of  
apolipoprotein, lipoprotein, or lipid associated  
therewith.

38. A method for purifying an  
apolipoprotein comprising  
reacting a solution containing  
apolipoprotein with an immobilized antibody selected  
from the group consisting of the anti-LDL monoclonal  
antibody produced by the hybridoma cell line HB<sub>3</sub>CB<sub>3</sub>,  
ATCC designation number HB 11612 and the anti-LDL  
RCB<sub>3</sub>M<sub>1</sub>D<sub>4</sub> recombinant antibody ATCC designation number  
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